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DEVELOPMENT OF A D DIMER BIOSENSOR TO MONITOR STROKE PATIENTS DURING THROMBOLYTIC THERAPY

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Introduction

In the United States, approximately 550,000 new cases of stroke are reported annually, resulting in 150,000 deaths and leaving 300,000 survivors disabled (1). The American Heart Association estimates that stroke and stroke-related disorders cost Americans between $15 to $30 billion annually, and the majority of the cost is attributed to the disabilities suffered by the survivors (2). Most strokes are caused by vascular occlusion due to cerebral atherosclerosis or due to thromboemboli of the extracranial or intracranial blood vessels. Thromboembolic strokes account for an estimated 300,000-400,000 of the 550,000 reported new cases of stroke each year (1). These thromboembolic strokes may be treatable by thrombolytic therapy.

Thrombolytic therapy is a minimally invasive procedure that involves using a steerable microguidewire to thread a microcatheter to the site of the occlusion. Pharmacological agents are then directly injected into the occlusion to lyse the clot. Fibrin Degradation Products (FDP) are produced when the pharmacological agent activates the proteolytic enzyme, plasmin, which attacks the fibrin chains of the clot by splitting peptide bonds thereby dissolving the thrombus into fragments. A particular diagnostic fragment is called the D dimer fragment which has antigenic properties. It consists of cross-linked gamma chain remnants of the fibrin molecule. The thrombolytic treatment is highly variable, i.e., different agents, infusion rates and dosages are used. However, optimal infusion rates, pharmocologic agents, dosage, and time for initiation of treatment have yet to be determined. Recognition of early warning signs of stroke followed by rapid intervention (i.e. thrombolytic treatments) may lower the number of disabilities suffered by the survivors, hence lowering the total costs (2).

We are developing a D dimer biosensor which may be of use for monitoring patients during thrombolytic therapy by accomplishing two goals. First, it can act as a diagnostic tool by indicating whether the occlusion is caused by atherosclerotic plaque or thrombus. Thrombolytic agents will not dissolve plaque, therefore if D dimer isn't detected after injection of the agent, then the occlusion is caused by an agent other than a thrombus and alternative therapy should be undertaken. Second, it could provide feedback on the dosage and infusion rate needed to lyse the clot. D dimer sensors could help eliminate the guesswork related to the dosage and infusion rate of the thrombolytic agents, providing surgeons with a faster diagnosis and treatment plan and reducing hemorrhaging incidents.

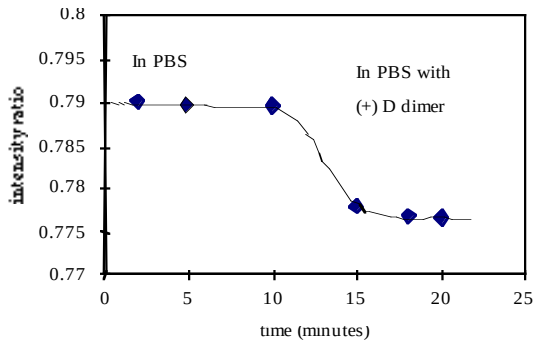
Sensor Fabrication and Optical System

For the sensing element, we immobilized D dimer monoclonal antibodies with a fluorescein isothiocyanate (FITC) label on the end of the 125 µm silica optical fiber using sol-gel dip coating methods. The D dimer concentration was 1.125 µM. The benefits of sol gel fiber optic biosensors have recently been discussed for other applications (3). For the D dimer sensor, a ratiometric-based system was utilized. Excitation was provided by a xenon halogen lamp. This light passed through a chopper wheel and then was split with some directed into a reference photodiode. The rest of the light passed through a biconvex lens and an interference filter. It was then reflected by a dichroic mirror. It passed through a second biconvex lens which coupled the light to the optical fiber. The emission fluorescence traveled back through the fiber and biconvex lens and was transmitted through the dichroic mirror, filtered and then coupled to a second photodiode. The photodiodes and chopper control were interfaced to lock-in amplifiers which were interfaced to a computer. A computer program then calculated the voltage ratio of the two photodiodes

Results and Discussion

Figure 1 shows the quenching of FITC fluorescence when the D dimer antibodies and antigens combined. Initially, the fiber tip was placed in a D dimer free phosphate buffer solution. A (+) D dimer antigen control was then added to the PBS. The concentration of D dimer antigen in PBS was 5 µg/ml which is the minimum detection level in standard *in vitro* diagnostic D dimer test kits. As the D dimer antibodies combine with the antigens, there is a quenching of the FITC fluorescence.

Figure 1. D dimer sensor



Initial indications are that the D dimer sensor is not completely reversible. This could be due to the high affinity between the D dimer antibodies and antigens. Studies are underway to improve the reversibility. Long-term viability studies are in the process of being evaluated. Currently the D dimer antibodies remain viable at least 30 days while encapsulated in the sol-gel network.

The D dimer sensor has demonstrated a response when in the presence of D dimer antigens. Studies are in process to improve and expand its performance.

References

1. JC Grotta, et al.; *Stroke: Clinical Updates*. 3:9 (1994)
2. LB Goldstein, et al.; *JAMA*. 271:1114 (1994)
3. S Braun, et al.; *Materials Letters*. 10:1 (1990)

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